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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US92/06681 <b>(22) International Filing Date:</b> 10 August 1992 (10.08.92)  <b>(30) Priority data:</b> 744,619 12 August 1991 (12.08.91) US  <b>(71) Applicant:</b> RESEARCH CORPORATION TECHNOLOGIES, INC. [US/US]; 6840 East Broadway Boulevard, Tucson, AZ 85710 (US).  <b>(72) Inventors:</b> HOUGHTON, Peter, J. ; 1718 Overton Park, Memphis, TN 38112 (US). HORTON, Julie, K. ; 11 Observation Court, #301, Germantown, MD 20876 (US). THIMMAIAH, Kuntebommanahalli, N. ; 1138 Lalithadri Road, II Cross, Kuyempunagar, Mysore-570023 (IN).		<b>(74) Agent:</b> SCOTT, Anthony, C.; Scully, Scott, Murphy & Presser, 400 Garden City Plaza, Garden City, NY 11530 (US).  <b>(81) Designated States:</b> CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> N-SUBSTITUTED PHENOXAZINES FOR TREATING MULTIDRUG RESISTANT CANCER CELLS  <b>(57) Abstract</b>  Phenoxazines, unsubstituted or N-substituted as defined herein, can potentiate the antitumor effectiveness of chemotherapeutic agents, particularly in multiple drug resistant (MDR) cells.		

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N-SUBSTITUTED PHENOXAZINES FOR  
TREATING MULTIDRUG RESISTANT CANCER CELLS

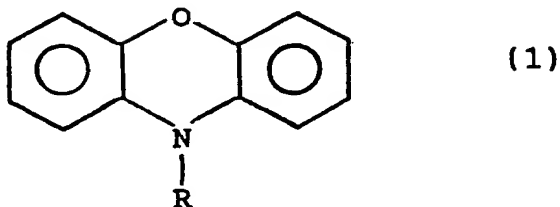
The present invention is directed to chemotherapy of cancer.

5 A major reason for failure of treatment of cancer patients is resistance to conventional chemotherapeutic agents. One type of drug resistance, called multi-drug resistance (MDR) is characterized by cross-resistance to functionally and structurally unrelated  
10 chemotherapy drugs, such as doxorubicin, vincristine (VCR), vinblastine (VLB), colchicine, and actinomycin D. A number of drugs appear to be active in modifying MDR in model systems, including the calcium channel blocker, verapamil (VRP), the calmodulin inhibitor,  
15 trifluoperazine, the anti-arrhythmic drug, quinidine, reserpine, cyclosporin A, Vinca alkaloid analogs, dihydropyridines, and pyridine analogs. Thus, it can be seen that agents that reverse MDR apparently do not seem to have common features. Although several of these MDR-  
20 reversing agents have been or are now being tested clinically in cancer patients, they have largely failed to enhance sensitivity to the chemotherapeutic agent. Instead, serious toxicities develop at or below plasma drug levels required for MDR reversal in vitro.

25 A tricyclic compound, phenoxazine, has been found to potentiate the uptake of VCR and VLB in MDR GC<sub>3</sub>/Cl and KBCh<sup>R</sup>-8-5 cells to a greater extent than verapamil. While this discovery has utility and holds promise, it would be desirable to identify derivatives  
30 of phenoxazine which would modulate MDR and which show even higher stability and lower toxicity.

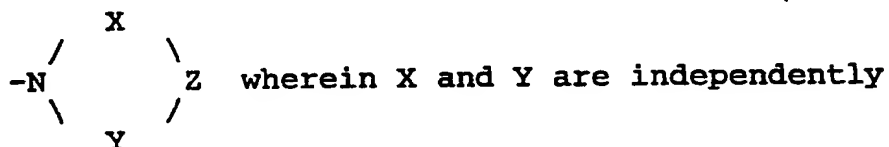
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1 In one aspect, the present invention comprises  
compounds of formula (1):



5 and pharmacologically acceptable salts thereof,  
wherein R is  $-\text{[C(O)]}_a-\text{(CH}_2\text{)}_b\text{-A}$ ; wherein a is 0 or 1 and  
10 b is an integer from 0 to 6, provided that a and b are  
not both zero;

A is selected from the group consisting of  
-NR<sub>1</sub>R<sub>2</sub> wherein R<sub>1</sub> and R<sub>2</sub> are independently  
alkyl having 1 to 4 carbon atoms, and either or both of  
15 R<sub>1</sub> and R<sub>2</sub> are optionally substituted with -OH;

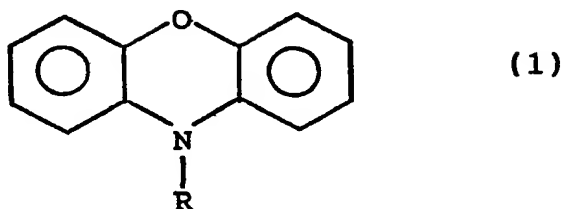


20 alkylene having 1 to 4 carbon atoms, and Z is -O-,  
-N(R<sub>3</sub>)- or -CH(R<sub>4</sub>)-, wherein R<sub>3</sub> is hydrogen or alkyl  
having 1 to 4 carbon atoms optionally substituted with a  
hydroxyl group, and wherein R<sub>4</sub> is hydrogen or alkyl  
having 1 to 4 carbon atoms optionally substituted with a  
25 hydroxyl groups;

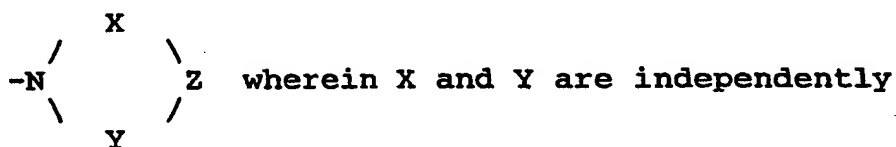
halide; and trihalomethyl.

The present invention also relates to a method  
of potentiating the cytotoxicity of an agent cytotoxic  
to a tumor cell, comprising administering to said tumor  
30 cell, while it is exposed to said cytotoxic agent, a  
potentiating agent in an amount effective to potentiate  
the cytotoxicity of said cytotoxic agent to said cell,

1 wherein said potentiating agent comprises a compound of  
 formula (1):



or a pharmacologically acceptable salt thereof,  
 wherein R is -H or  $-\text{[C(O)]}_a-\text{(CH}_2\text{)}_b\text{-A}$ ;  
 10 wherein a is 0 or 1 and b is an integer from 0 to 6,  
 provided that a and b are not both zero; and  
 A is selected from the group consisting of  
 $-\text{NR}_1\text{R}_2$  wherein  $\text{R}_1$  and  $\text{R}_2$  are independently  
 alkyl having 1 to 4 carbon atoms, and either or both of  
 15  $\text{R}_1$  and  $\text{R}_2$  are optionally substituted with -OH;



20 alkylene having 1 to 4 carbon atoms, and Z is -O-, -  
 $\text{N(R}_3\text{)-}$  or  $-\text{CH(R}_4\text{)-}$ , wherein  $\text{R}_3$  is hydrogen or alkyl  
 having 1 to 4 carbon atoms optionally substituted with a  
 hydroxyl group, and wherein  $\text{R}_4$  is hydrogen or alkyl  
 having 1 to 4 carbon atoms optionally substituted with a  
 25 hydroxyl group;  
 halide; and trihalomethyl.

The present invention further relates to a  
 composition comprising cytotoxic agent toxic to tumor  
 cells, and a potentiating agent which potentiates the  
 30 cytotoxicity of said cytotoxic agent, wherein said  
 potentiating agent comprises a compound of formula (1)  
 and wherein said cytotoxic agent and potentiating agent

are present in amounts effective to render the composition cytotoxic to tumor cells.

The present invention still further relates to a method of killing a tumor cell which comprises administering to said cell a composition as described above in an amount effective to kill said cell.

As described in more detail below, the present invention provides novel and effective means for potentiating the desired cytotoxic effect of anticancer drugs in tumor cells and especially in multidrug-resistant (MDR) cells.

One preferred group of compounds of the formula (1) is the N-alkyl derivatives, in which a is 0 in formula (1). Of those compounds wherein a is 0, the more preferred include those in which b is 3 or 4, denoting unbranched propylene and butylene moieties; R<sub>1</sub> and R<sub>2</sub> each are ethyl, n-propyl, ω-hydroxyethyl, or ω-hydroxypropyl; X and Y are each -CH<sub>2</sub>- or -CH<sub>2</sub>CH<sub>2</sub>- and, more preferably, both X and Y are -CH<sub>2</sub>CH<sub>2</sub>-; and R<sub>3</sub> and R<sub>4</sub> are each -H or ethyl, propyl, e.g. n-propyl, ω-hydroxyethyl or ω-hydroxypropyl. Other more preferred embodiments when a is 0 are those derivatives wherein b is 3 or 4 and A is halogen, preferably chloro.

Another preferred group of compounds of formula (1) is the N-acyl derivatives, in which a is 1 in formula (1). Of those compounds wherein a is 1, the more preferred include those in which b is 1 or 2, more preferably 1; R<sub>1</sub> and R<sub>2</sub> are each ethyl, n-propyl, ω-hydroxyethyl or ω-hydroxypropyl; X and Y are each -CH<sub>2</sub>- or -CH<sub>2</sub>CH<sub>2</sub>-, and more preferably, both X and Y are -CH<sub>2</sub>CH<sub>2</sub>-; each of R<sub>3</sub> and R<sub>4</sub> is -H or ethyl, n-propyl, ω-hydroxyethyl or ω-hydroxypropyl. Other more preferred

1 embodiments are those in which b is 0 or 1 and A is trihalomethyl, preferably trichloromethyl or trifluoromethyl; and in which b is 1 or 2 and A is halogen, preferably chloro.

5 As used herein, unless specified otherwise, "alkyl" means saturated, branched or unbranched groups of the formula  $-(C_nH_{2n+1})$ ; "halo" or "halogen" means fluoro, chloro, bromo, and/or iodo; and the optional hydroxyl and halo substituents disclosed herein can be  
10 on any carbon of an alkyl or alkylene group.

The compounds of this invention form salts, which are also within the scope of the invention, with various inorganic and organic acids. The pharmacologically acceptable acid addition salts of the  
15 compounds of the present invention may be prepared by conventional means, such as by reacting with an appropriate acid providing the desired anion, either in a solvent or medium in which the salt is insoluble, or in water. The salts of strong acids are preferred. As  
20 exemplary, but not limiting, of pharmacologically acceptable acid salts are the salts of hydrochloric, hydrobromic, sulfuric, nitric, acetic, fumaric, malic, maleic, tartaric and citric acids.

In general, the synthesis of the N-alkyl and N-acyl derivatives is straightforward. N-alkylation can  
25 be achieved in the presence of basic condensing agents like sodium amide. The general procedure for preparing the N-alkyl derivatives of formula (1) consists of the condensation of phenoxazine with the appropriate  $\alpha$ ,  $\omega$ -di-alkylhalide in such as  $Cl-(CH_2)_b-Br$  wherein b is 1 to  
30 6, in the presence of sodium amide, either in liquid ammonia or in an anhydrous solvent such as toluene or



1 benzene. For instance, the reaction of phenoxazine with  
mixed chlorobromoalkanes in the presence of sodium amide  
gives reactive N-chloroalkylphenoxazines, which can then  
be converted to the desired compound by reaction with  
5 an intermediate of the formula  $H-(CH_2)_b-A$  wherein b and  
A have the meanings set forth above.

More specifically, compounds such as those  
described in Examples 1-14 below can be prepared by  
first alkylating phenoxazine with 1-bromo-3-  
10 chloropropane or 1-bromo-4-chloropropane to produce 10-  
(3'-chloropropyl) phenoxazine or 10-(4'-  
chlorobutyl)phenoxazine, alkylation being accomplished  
by first converting phenoxazine to the anionic species  
using the strong base, sodium amide. Iodide-catalyzed  
15 nucleophilic substitution of the propyl or butyl  
chloride with various secondary amines (e.g. N,N-  
diethylamine, N,N-diethanolamine, morpholine,  
piperidine, pyrrolidine and 8-hydroxyethyl-piperazine)  
by refluxing for about 20 hours with potassium carbonate  
in anhydrous acetonitrile affords the free bases of  
20 formula (1).

The acyl derivatives of formula (1) can be  
synthesized by acylating phenoxazine with a compound of  
the formula  $Cl-C(O)-C(CH_2)_{o-s}-Cl$  and then reacting the  
product with an amine of the formula H-A, wherein A has  
25 the meaning given above in anhydrous acetonitrile  
containing potassium iodide. The haloacetylphenoxazine  
can be prepared by reacting phenoxazine with the  
anhydride  $(C(halo)_sCO)_2O$ .

30 All the compounds described in Examples 1-14  
were separated and purified by column chromatography or  
recrystallization and dried under high vacuum. The

1 structures were established by UV-, IR,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR  
and EIMS spectral data, and by elemental analyses. The  
physical properties of the compounds are given in Table  
I. The UV-spectral data of N-substituted phenoxazines  
5 are in close agreement with the spectral characteristics  
of analogous heterocycles. The IR bands also indicate  
the presence of characteristic functional groups, and  
peaks at  $1670\text{--}1695\text{ cm}^{-1}$  indicated the presence of  $>\text{C}=\text{O}$   
group in the acyl derivatives. The  $^1\text{H}$ -NMR in  $\text{CDCl}_3$ ,  
10 typical of phenoxazine compound, showed eight aromatic  
protons and the data are in accordance with the  
structures assigned. The assignment of protons is fully  
supported by the integration curves. The  $^{13}\text{C}$ -NMR  
spectrum of each N-substituted phenoxazine exhibited  
15 size signals representing 12 aromatic carbons. The GC-  
Mass spectrum showed an intense molecular ion peak ( $\text{M}^+$ )  
for each of the compounds characteristic of the  
phenoxazine type of structure. The spectral data are  
consistent with the assigned structures.

#### 20 SYNTHESIS AND ANALYSIS

In the syntheses and experiments described  
below, melting points were recorded on a Perkin-Elmer  
Model 1320 spectrophotometer, as KBr pellets; UV-spectra  
were recorded in MeOH on a Perkin-Elmer Lambda 3B  
spectrophotometer. Elemental analyses were performed  
25 and found values within 0.4% of theoretical, unless  
otherwise noted. Reactions were monitored by tlc. For  
tlc, Analtech silica gel GF plates (20 x 20 cm, 250  
microns, glass-backed), with petroleum ether-  
ethylacetate (9.7:0.3 by volume, system A), and  
30 ethylacetate-methanol (9.9:0.1 by volume, system B) as  
solvents were used. Column chromatography utilized

1 silica gel Merc grade 60 (230-400 mesh, 60Å). <sup>1</sup>H- and  
1 <sup>13</sup>C-NMR spectra were recorded in CDCl<sub>3</sub> solution in a 5-  
mm tube on an IBM NR 200 AF Fourier transform  
spectrometer with tetramethylsilane as internal  
5 standard. Chemical shifts are expressed as "δ" (ppm)  
5 values. The spectrometer was internally locked to the  
deuterium frequency of the solvent. Electron-impact  
mass spectra (EIMS) were recorded on a Ribermag R10-10C  
GC-mass spectrometer with an upper mass limit of 1500  
10 AMU. All chemicals and supplies were obtained from  
10 standard commercial sources unless otherwise indicated.  
Phenoxazine, secondary amines indicated in the text, and  
anhydrous organic solvents were purchased from Aldrich  
Chemical Co. (Milwaukee, WI). Vincristine sulfate  
15 (oncovin) was purchased from Eli Lilly and Co.  
15 (Indianapolis, IN), and vinblastine sulfate was from  
Cetus Corporation (Emeryville, CA). [G-<sup>3</sup>H]vincristine  
(sp. act. (specific activity) 7.1 Ci/mmol), and [G-  
<sup>3</sup>H]vinblastine (sp. act. 10.1 Ci/mmol) were obtained  
20 from Amersham Corporation (Arlington Heights, IL).  
20 Verapamil hydrochloride, colchicine, RPMI-1640 medium,  
powder with glutamine and without sodium bicarbonate  
were purchased from the Sigma Chemical Co. (St. Louis,  
MO).

25 The synthesis of representative compounds of  
25 formula (1) is described below. Each of the indicated  
compounds in these Examples is considered a preferred  
embodiment of the present invention.

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EXAMPLE 1

1           10-(3'-chloropropyl)-phenoxazine. To a  
suspension of sodium amide (1.72 g) in 100 ml of liquid  
ammonia, 7g (0.038 mol) of phenoxazine was added. After  
5 stirring for 30 minutes, 6.3 g (0.04 mol., 3.96 mL) of  
1-bromo-3-chloropropane was added slowly with constant  
stirring. After one more hour, ammonia was allowed to  
evaporate and solid ice pieces were added carefully  
followed by cold water. When the reaction ceased, the  
10 mixture was extracted three times with ether. The ether  
solution was washed three times with water, dried over  
anhydrous sodium sulfate and evaporated. The residue  
was chromatographed on silica gel. Petroleum ether-  
ethylacetate (9 mL + 3 mL) eluted the pure title  
15 compound (7.94 g) as white crystals.  $\lambda_{max}$  (MeOH)  
218, 238 and 321 nm; IR (KBr) 3070, 2860, 1630, 1490,  
1380, 1275, 920, 815 and 740  $cm^{-1}$ ;  $^1H$ -NMR ( $\delta$ ) 6.47-6.82  
(m, 8H, ArH,  $H_1$ - $H_4$  and  $H_6$ - $H_9$ ), 2.11 (m, 2H,  $H_1$ ), 3.63  
(m, 2H,  $H_x$ ), and 3.69 (m, 2H,  $H_m$ );  $^{13}C$ -NMR ( $^1H$   
20 decoupled) 111.23 ( $C_1$  and  $C_9$ ), 115.50 ( $C_4$  and  $C_6$ ),  
121.07 ( $C_3$  and  $C_7$ ), 123.70 ( $C_2$  and  $C_8$ ), 133.03 ( $C_1$  and  
 $C_9$ ), 144.92 ( $C_4$  and  $C_6$ ), 27.82 ( $C_1$ ), 41.09 ( $C_x$ ) and  
42.63 ( $C_m$ ); EIMS (m/z) 259 ( $M^+$ ).

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EXAMPLE 2

1                   10-(3'-diethylaminopropyl)phenoxazine. 1g  
(4.31 mmol) of the product of Example 1 was dissolved in  
150 mL of anhydrous acetonitrile, and 1.5 g KI, 2.13 g  
5  $K_2CO_3$  and 1.6 mL (15.4 mmol) of N,N-diethylamine were  
added. The mixture was refluxed overnight until a  
substantial amount of product was formed (TLC, System B,  
 $R_f = 0.40$ ). The reaction mixture was diluted with water  
and extracted with ether three times. The ether layer  
10 was washed with water and dried over anhydrous  $Na_2SO_4$   
and evaporated. The crude oil was subjected to column  
chromatography for purification. Ethylacetate-petroleum  
ether (50 mL + 50 mL) eluted the title compound as the  
free base as a colorless oil, which was dried and used  
15 for NMR studies. An ethereal solution of the free base  
was treated with an excess of tartaric acid to separate  
the hygroscopic tartrate salt (1.2 g). UV- $\lambda_{max}$  (MeOH)  
215, 238 and 320 nm; IR ( $CHCl_3$ ) 3378, 2974, 2838, 1453,  
1375, 1155, 973 and 722  $cm^{-1}$ ;  $^1H$ -NMR (' $\delta$ ') 6.51-6.80 (m,  
8H, ArH,  $H_1$ - $H_4$  and  $H_6$ - $H_9$ ), 1.16 (t, 6H,  $H_c$  and  $H_d$ ), 1.70  
20 (m, 2H,  $H_1$ ), 2.50 (q, 4H,  $H_a$  and  $H_b$ ,  $J=7$  Hz), 3.42-3.63  
(m, 4H,  $H_x$  and  $H_m$ );  $^{13}C$ -NMR 111.54 ( $C_1$  and  $C_9$ ), 115.49  
( $C_4$  and  $C_6$ ), 121.21 ( $C_3$  and  $C_7$ ), 123.85 ( $C_2$  and  $C_8$ ),  
132.72 ( $C_{10}$  and  $C_5$ ), 144.95 ( $C_4$  and  $C_6$ ), 8.21 ( $C_c$  and  
 $C_d$ ), 19.90 ( $C_1$ ), 40.72 ( $C_a$  and  $C_b$ ), 45.87 ( $C_m$ ), and  
25 48.50 ( $C_x$ ); EIMS (m/z) 296 ( $M^+$ ).

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EXAMPLE 3

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10-(3'-bishydroxyethylaminopropyl)phenoxazine.

The procedure used for Example 2 was repeated with 1g, (4.31 mmol) of the product of Example 1, 1.5 g KI, and 1.62 g (15.4 mmol, 1.5 mL) of diethanolamine.

5

Recrystallization of the solid in ethylacetate and petroleum ether gave (1.14 g) of the title compound in the pure form. UV- $\lambda_{max}$  (MeOH) 218, 239, and 322 nm; IR (KBr) 3300, 2960, 2880, 1590, 1490, 1440, 1375, 1270, 1190, 1125, 1075, 1040, 890, 840, and 740  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\delta$ ) 6.44-6.78 (m, 8H, ArH,  $\text{H}_1$ - $\text{H}_4$  and  $\text{H}_6$ - $\text{H}_9$ ), 1.71-1.82 (m, 2H,  $\text{H}_1$ ), 2.54-2.61 (t, 4H,  $\text{H}_a$  and  $\text{H}_b$ ,  $J = 6$  Hz), 3.39 - 3.68 (m, 8H,  $\text{H}_k$ ,  $\text{H}_c$ , and  $\text{H}_d$  and  $\text{H}_m$ ), and 2.95 (s,  $\text{H}_e$  and  $\text{H}_f$ , disappearing on  $\text{D}_2\text{O}$  exchange);  $^{13}\text{C-NMR}$  111.37 ( $\text{C}_1$  and  $\text{C}_9$ ), 115.33 ( $\text{C}_4$  and  $\text{C}_6$ ), 120.80 ( $\text{C}_3$  and  $\text{C}_7$ ), 123.66 ( $\text{C}_2$  and  $\text{C}_8$ ), 133.25 ( $\text{C}_1$  and  $\text{C}_9$ ), 144.99 ( $\text{C}_4$  and  $\text{C}_6$ ), 22.42 ( $\text{C}_1$ ), 41.83 ( $\text{C}_a$  and  $\text{C}_b$ ), 52.38 ( $\text{C}_m$ ), 55.91 ( $\text{C}_k$ ) and 59.64 ( $\text{C}_c$  and  $\text{C}_d$ ); EIMS ( $m/z$ ) 328 ( $\text{M}^+$ ).

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EXAMPLE 4

1                   10-(3'-N-morpholinopropyl)phenoxazine. The  
                  procedure used for Example 2 was repeated with 1g of the  
                  product of Example 1, 1.5 g KI, 2.0 g K<sub>2</sub>CO<sub>3</sub> and 1.4 g  
5                   (15.40 mmol, 1.34 mL) of morpholine. The oily residue  
                  was purified by column chromatography to give the title  
                  compound as a brown oil. An ethereal solution of the  
                  free base was treated with ethereal hydrochloride to  
                  give the hydro-chloride salt (1.07 g). UV- $\lambda_{max}$  (MeOH)  
10                   216, 239, and 320 nm; IR (KBr) 3200, 1495, 1380, 1280,  
                  1230, 1135, 1100, 1050, 1020, 980, 870, 830, 760 and 735  
                  cm<sup>-1</sup>; <sup>1</sup>H-NMR (' $\delta$ ') 6.63-6.81 (m, 8H, ArH, H<sub>1</sub>-H<sub>4</sub> and H<sub>6</sub>-  
                  H<sub>9</sub>), 1.78 (m, 2H, H<sub>1</sub>), 2.40 (t, 4H, H<sub>a</sub> and H<sub>b</sub>, J = 12  
                  Hz), 3.45-3.80 (m, 8H, K<sub>x</sub>, H<sub>m</sub>, H<sub>c</sub> and H<sub>d</sub>); <sup>13</sup>C-NMR  
15                   111.64 (C<sub>1</sub> and C<sub>9</sub>), 115.80 (C<sub>4</sub> and C<sub>6</sub>), 121.59 (C<sub>3</sub> and  
                  C<sub>7</sub>), 123.91 (C<sub>2</sub> and C<sub>8</sub>), 133.50 (C<sub>1</sub> and C<sub>9</sub>), 145.11  
                  (C<sub>4</sub> and C<sub>6</sub>), 20.06 (C<sub>1</sub>), 40.93 (C<sub>a</sub> and C<sub>b</sub>), 51.91  
                  (C<sub>m</sub>), 55.20 (C<sub>x</sub>), and 63.50 (C<sub>c</sub> and C<sub>d</sub>); EIMS (m/z) 310  
                  (M<sup>+</sup>).

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EXAMPLE 5

1                   10-(3'-N-pip ridinopropyl)ph noxazine. Th  
                  procedure used for Example 2 was used with 1.12 g (4.31  
                  mmol) of the product of Example 1, 1.5 g IH, 2.4 g K<sub>2</sub>CO<sub>3</sub>  
5                   and 1.5 g (17.62 mmol, 1.74 mL) of piperidine. The  
                  product was chromatographed on silica gel with petroleum  
                  ether-ethylacetate (1:1 by volume) to obtain the pure  
                  title compound in the form of an oil. By adding  
                  ethereal hydrochloride to the ether solution of the free  
10                  base, the hydrochloride salt (1.15 g) was obtained. UV-  
                  λ<sub>max</sub> (MeOH) 218, 238 and 320 nm; IR (KBr) 3300, 2940,  
                  2680, 1595, 1495, 1385, 1275, 1160, 1050, 825 and 745  
                  cm<sup>-1</sup>; <sup>1</sup>H-NMR ('δ') 6.56-6.86 (m, 8H, ArH, H<sub>1</sub>-H<sub>4</sub> and H<sub>6</sub>-  
                  H<sub>9</sub>), 1.53 (m, 6H, H<sub>a</sub>, H<sub>d</sub> and H<sub>e</sub>), 2.30 (m, 2H, H<sub>1</sub>),  
                  2.56-2.67 (m, 4H, H<sub>a</sub> and H<sub>b</sub>), and 3.45-3.70 (m, 4H, H<sub>x</sub>  
15                  and H<sub>m</sub>); <sup>13</sup>C-NMR 111.65 (C<sub>1</sub> and C<sub>9</sub>), 115.62 (C<sub>4</sub> and C<sub>6</sub>),  
                  121.38 (C<sub>3</sub> and C<sub>7</sub>), 123.88 (C<sub>2</sub> and C<sub>8</sub>), 132.73 (C<sub>1</sub>. and  
                  C<sub>9</sub>.), 144.98 (C<sub>4</sub>. and C<sub>6</sub>.), 20.21 (C<sub>e</sub>), 21.93 (C<sub>e</sub> and  
                  C<sub>d</sub>), 22.50 (C<sub>1</sub>), 41.05 (C<sub>a</sub> and C<sub>b</sub>), 53.18 (C<sub>m</sub>), and  
20                  54.62 (C<sub>x</sub>); EIMS (m/z) 308 (M<sup>+</sup>).

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EXAMPLE 6

1                   10-(3'-8-hydroxyethylpiperazinopropyl)  
phenoxazine. The procedure used for Example 2 was  
repeated with 1 g (4.31 mmol) of the product of Example  
1, 1.5 g KI, 2.12 g K<sub>2</sub>CO<sub>3</sub> and 2 g (15.4 mmol, 1.9 mL) of  
5 8-hydroxyethylpiperazine. The free base was  
recrystallized in petroleum ether-ether mixture (7:3 by  
volume) to give 1.16 g of the title compound. UV- $\lambda_{max}$   
(MeOH) 217, 239 and 322 nm; IR (KBr) 3060, 2820, 1630,  
1595, 1495, 1385, 1270, 1160, 1070, 980, 850, 810 and  
10 735 cm<sup>-1</sup>; <sup>1</sup>H-NMR (' $\delta$ ') 6.46-6.76 (m, 8H, ArH, H<sub>1</sub>-H<sub>4</sub> and  
H<sub>6</sub>-H<sub>9</sub>), 1.74 (m, 2H, H<sub>1</sub>), 2.33-2.80 (M, 12H, H<sub>a</sub> and H<sub>b</sub>,  
H<sub>c</sub> and H<sub>d</sub>, H<sub>e</sub> and H<sub>m</sub>), 2.79 (s, 1H, H<sub>g</sub>, disappearing on  
D<sub>2</sub>O exchange), 3.47-3.65 (m, 4H, H<sub>x</sub> and H<sub>z</sub>); <sup>13</sup>C-NMR  
111.34 (C<sub>1</sub> and C<sub>9</sub>), 115.24 (C<sub>4</sub> and C<sub>6</sub>), 120.66 (C<sub>3</sub> and  
15 C<sub>7</sub>), 123.50 (C<sub>2</sub> and C<sub>8</sub>), 133.30 (C<sub>1</sub> and C<sub>9</sub>), 144.83  
(C<sub>4</sub> and C<sub>6</sub>), 22.58 (C<sub>1</sub>), 41.72 (C<sub>m</sub>), 52.96 (C<sub>a</sub> and  
C<sub>b</sub>), 53.28 (C<sub>c</sub> and C<sub>d</sub>), 55.19 (C<sub>x</sub>); 57.77 (C<sub>e</sub>), and  
59.34 (C<sub>z</sub>); MS (m/z) 353 (M<sup>+</sup>).

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EXAMPLE 7

1                   10-(3'-N-pyrrolidinopropyl)ph noxazine. The  
procedure used for Example 2 was repeated with 1g of the  
title product of Example 1, 1.5 g KI, 2g K<sub>2</sub>CO<sub>3</sub> and 1.1g  
5 (15.5 mmol, 1.3 mL) of pyrrolidine. The product was  
purified by column chromatography and the oil was  
converted into the hydrochloride salt (1.02g). UV- $\lambda_{max}$   
(MeOH) 217, 239, and 319 nm; IR (KBr) 3300, 2660, 1590,  
1490, 1375, 1270, 1130, 920, 820 and 745 cm<sup>-1</sup>; <sup>1</sup>H-NMR  
10 (' $\delta$ ') 6.46-6.77 (m, 8H, ArH, H<sub>1</sub>-H<sub>4</sub> and H<sub>6</sub>-H<sub>9</sub>), 2.01-2.17  
(t, 4H, H<sub>a</sub> and H<sub>d</sub>, J = 13 Hz), 2.21 (m, 2H, H<sub>1</sub>), 3.06-  
3.14 (t, 4H, H<sub>a</sub> and H<sub>b</sub>), and 3.60-3.67 (m, 4H, H<sub>k</sub> and  
H<sub>m</sub>); <sup>13</sup>C-NMR 111.60 (C<sub>1</sub> and C<sub>9</sub>), 115.66 (C<sub>4</sub> and C<sub>6</sub>),  
121.40 (C<sub>3</sub> and C<sub>7</sub>), 123.85 (C<sub>2</sub> and C<sub>8</sub>), 132.73 (C<sub>1</sub> and  
C<sub>9</sub>), 144.98 (C<sub>4</sub> and C<sub>6</sub>), 22.25 (C<sub>a</sub> and C<sub>d</sub>), 23.30  
15 (C<sub>1</sub>), 40.90 (C<sub>a</sub> and C<sub>b</sub>), 52.80 (C<sub>m</sub>), and 53.63 (C<sub>k</sub>); MS  
(m/z) 294 (M<sup>+</sup>).

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EXAMPLE 8

1           10-(4'-chlorobutyl)phenoxazine, (8.4 g) in the  
pure form was prepared following the procedure used for  
Example 1 with 7g phenoxazine, 1.63 g sodium amide and  
4.36 mL of 1-bromo-4-chlorobutane (0.038 mol) to produce  
5 the title compound. UV- $\lambda_{max}$  (MeOH) 200, 212, 238, and  
320 nm; IR (KBr) 3060, 2980, 1630, 1590, 1495, 1380,  
1280, 1130, 915, 840 and 730  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (' $\delta$ ') 6.36-  
6.74 (m, 8H, ArH,  $\text{H}_1\text{-H}_4$  and  $\text{H}_6\text{-H}_9$ ), 1.75 (broad, 4H,  $\text{H}_1$   
and  $\text{H}_m$ ), and 3.38-3.50 (m, 4H,  $\text{H}_k$  and  $\text{H}_n$ ),  $^{13}\text{C-NMR}$   
10 111.43 ( $\text{C}_1$  and  $\text{C}_9$ ), 115.53 ( $\text{C}_4$  and  $\text{C}_6$ ), 121.01 ( $\text{C}_3$  and  
 $\text{C}_7$ ), 123.83 ( $\text{C}_2$  and  $\text{C}_8$ ), 133.27 ( $\text{C}_1$  and  $\text{C}_9$ ), 145.10  
( $\text{C}_4$  and  $\text{C}_6$ ), 22.60 ( $\text{C}_m$ ), 29.87 ( $\text{C}_1$ ), 43.27 ( $\text{C}_k$ ), and  
44.61 ( $\text{C}_n$ ); EIMS (m/z) 273 ( $\text{M}^+$ ).

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EXAMPLE 9

1                   10-(4'-di thylaminobutyl)phenoxazine. The  
procedure used for Example 2 was followed with 1g (3.65  
mmol) of the product of Example 8, 1.5g KI, 2g K<sub>2</sub>CO<sub>3</sub> and  
5 1.07 g (14.63 mmol, 1.5 mL) of N,N-diethylamine to  
obtain the indicated product. The oily product was  
chromato-graphed on the silica gel with CH<sub>3</sub>OH-CHCl<sub>3</sub>  
(3:1) and the hydrochloride salt (.076g) was obtained in  
the pure form. UV- $\lambda_{max}$  (MeOH) 201, 213, 239 and 320 nm;  
10 IR (KBr) 3300, 2940, 1590, 1495, 1380, 1270, 1130, 1040,  
925 and 750 cm<sup>-1</sup>; <sup>1</sup>H-NMR (' $\delta$ ') 6.47-6.80 (m, 8H, ArH,  
H<sub>1</sub>-H<sub>4</sub> and H<sub>6</sub>-H<sub>9</sub>), 1.33 (broad, 6H, H<sub>c</sub> and H<sub>d</sub>), 1.66-1.91  
(m, 4H, H<sub>1</sub> and H<sub>m</sub>), 3.05 (very broad, 6H, H<sub>a</sub>, H<sub>b</sub> and  
H<sub>n</sub>), and 3.50 (m, 2H, H<sub>k</sub>); <sup>13</sup>C-NMR 111.51 (C<sub>1</sub> and C<sub>9</sub>),  
115.31 (C<sub>4</sub> and C<sub>6</sub>), 120.99 (C<sub>3</sub> and C<sub>7</sub>), 123.75 (C<sub>2</sub> and  
15 C<sub>8</sub>), 132.78 (C<sub>1</sub> and C<sub>9</sub>), 144.78 (C<sub>4</sub> and C<sub>6</sub>), 8.54  
(C<sub>c</sub> and C<sub>d</sub>), 21.02 (C<sub>m</sub>), 22.46 (C<sub>1</sub>), 43.05 (C<sub>a</sub> and C<sub>b</sub>),  
46.50 (C<sub>n</sub>), and 51.26 (C<sub>k</sub>); MS (m/z) 310 (M<sup>+</sup>).

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EXAMPLE 10

1           10-(4'-bishydroxyethylaminobutyl) phenoxazine,  
as its hydrochloride salt (1.11g) was obtained by  
following the procedure of Example 3 with 1g of the  
product of Example 8, 1.5g KI and 1.54 g (14.65 mmol,  
5 1.4 mL) of N,N-diethanolamine followed by column  
chromato-graphy. UV- $\lambda_{max}$  (MeOH) 204, 210, 238 and 321  
nm; IR (KBr) 3280, 2850, 1630, 1590, 1490, 1375, 1270,  
1135, 1095, 1065, 1045, 1020, 925, 890, 845, and 740  $\text{cm}^{-1}$ ;  
10  $^1\text{H-NMR}$  (' $\delta$ ') 6.52-6.84 (m, 8H, ArH,  $\text{H}_1$ - $\text{H}_4$  and  $\text{H}_6$ - $\text{H}_9$ ),  
1.70-1.98 (m, 4H,  $\text{H}_1$ , and  $\text{H}_m$ ), 3.35-3.57 (broad, 10H,  
 $\text{H}_a$ ,  $\text{H}_b$ ,  $\text{H}_n$ ,  $\text{H}_k$ ,  $\text{H}_e$  and  $\text{H}_f$ ), 3.95 (t, 4H,  $\text{H}_c$  and  $\text{H}_d$ ; J =  
7 Hz), and 10.3 ( $\text{H}^+$ );  $^{13}\text{C-NMR}$  110.53 ( $\text{C}_1$  and  $\text{C}_9$ ), 114.17  
( $\text{C}_4$  and  $\text{C}_6$ ), 119.83 ( $\text{C}_3$  and  $\text{C}_7$ ), 122.76 ( $\text{C}_2$  and  $\text{C}_8$ ),  
131.85 ( $\text{C}_{10}$  and  $\text{C}_5$ ), 143.60 ( $\text{C}_4$  and  $\text{C}_6$ ), 19.98 ( $\text{C}_m$ ),  
15 21.10 ( $\text{C}_1$ ), 42.06 ( $\text{C}_n$ ), 52.92 ( $\text{C}_a$  and  $\text{C}_b$ ), 54.78 ( $\text{C}_k$ ),  
and 54.96 ( $\text{C}_e$  and  $\text{C}_d$ ); EIMS (m/z) 342 ( $\text{M}^+$ ).

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EXAMPLE 11

1                   10-(4'-N-morpholinobutyl)phenoxazine. The  
procedure used for Example 4 was repeated with 1 g of  
the product of Example 8, 1.5g KI, 2g of K<sub>2</sub>CO<sub>3</sub> and 1.273  
5 g (14.61 mmol, 1.3 mL) of morpholine. The product was  
recrystallized in ether-petroleum ether mixture (3:1) to  
give the title compound (0.95g). UV- $\lambda_{\text{max}}$  202, 213,  
239, and 321 nm; IR (KBr) 2960, 2810, 1630, 1595, 1495,  
1380, 1295, 1220, 1130, 1070, 1010, 970, 920, 870, 855,  
10 825, 765 and 745 cm<sup>-1</sup>; <sup>1</sup>H-NMR (' $\delta$ ') 6.53-7.29 (m, 8H,  
ArH, H<sub>1</sub>-H<sub>4</sub> and H<sub>6</sub>-H<sub>9</sub>), 1.61-1.74 (m, 4H, H<sub>1</sub> and H<sub>m</sub>),  
2.40-2.50 (m, 6H, H<sub>a</sub>, H<sub>b</sub>, and H<sub>n</sub>), 3.49 (m, 2H, H<sub>x</sub>), and  
3.49-3.78 (t, 4H, H<sub>c</sub> and H<sub>d</sub>, J = 12 Hz); <sup>13</sup>C-NMR 111.28  
(C<sub>1</sub> and C<sub>9</sub>), 115.28 (C<sub>4</sub> and C<sub>6</sub>), 120.67 (C<sub>3</sub> and C<sub>7</sub>),  
123.52 (C<sub>2</sub> and C<sub>8</sub>), 133.30 (C<sub>1</sub> and C<sub>9</sub>), 144.99 (C<sub>4</sub>.  
15 and C<sub>6</sub>), 22.34 (C<sub>m</sub>), 23.50 (C<sub>1</sub>), 43.63 (C<sub>n</sub>), 53.67 (C<sub>a</sub>  
and C<sub>b</sub>), 57.91 (C<sub>x</sub>), and 66.97 (C<sub>c</sub> and C<sub>d</sub>); EIMS (m/z)  
324 (M<sup>+</sup>).

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EXAMPLE 12

1                   10-(4'-N-piperidinobutyl)phenoxazine. 1g of  
the product of Example 8, 1.5g of KI, 2g K<sub>2</sub>CO<sub>3</sub> and 1.45g  
17.03 mmol, 1.5 mL) of piperidine were refluxed and  
5 processed according to the procedure used for Example  
10. Purification by column chromatography afforded the  
free amine as a brown oil which was converted into the  
hydrochloride salt (1.18 g). UV- $\lambda_{max}$  203, 210, 238, and  
320 nm; IR (KBr) 3320, 2940, 1625, 1590, 1490, 1380,  
1270, 1130, 1060, 955, 840, 820, and 730 cm<sup>-1</sup>; <sup>1</sup>H-NMR  
10 (' $\delta$ ') 6.42-6.81 (m, 8H, ArH, H<sub>1</sub>-H<sub>4</sub> and H<sub>6</sub>-H<sub>9</sub>), 1.44-1.82  
(m, 6H, H<sub>c</sub>, H<sub>d</sub> and H<sub>e</sub>), 1.98-21.8 (m, H<sub>1</sub> and H<sub>m</sub>), 2.70-  
2.97 (m, 4H, H<sub>a</sub> and H<sub>b</sub>), 3.39-3.45 (m, 4H, H<sub>x</sub> and H<sub>n</sub>)  
and 11.54 (H<sup>+</sup>); <sup>13</sup>C-NMR 111.42 (C<sub>1</sub> and C<sub>9</sub>), 115.32 (C<sub>4</sub>  
and C<sub>6</sub>), 120.98 (C<sub>3</sub> and C<sub>7</sub>), 123.71 (C<sub>2</sub> and C<sub>8</sub>), 132.78  
15 (C<sub>1</sub> and C<sub>9</sub>), 144.73 (C<sub>4</sub> and C<sub>6</sub>), 20.96 (C<sub>e</sub>), 21.79  
(C<sub>e</sub> and C<sub>d</sub>), 22.48 (C<sub>1</sub> and C<sub>m</sub>), 43.08 (C<sub>a</sub> and C<sub>b</sub>), 52.91  
(C<sub>n</sub>), and 56.70 (C<sub>x</sub>); EIMS (m/z) 322 (M<sup>+</sup>).

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EXAMPLE 13

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10-(4'- $\beta$ -hydroxyethylpiperazinobutyl)

phenoxazine. The procedure used for Example 6 was repeated with 1 g of the product of Example 8, 1.5g KI, and 1.9g (14.6 mmol, 1.8 mL) of  $\beta$ -hydroxyethylpiperazine. The oily residue was treated with 500  $\mu$ l of ethylacetate first and then with petroleum ether (20 mL), when a white crystalline solid separated out. The solid was recrystallized to give the pure title compound (1.21g). UV- $\lambda_{max}$  (MeOH) 202, 239, and 320 nm; IR (KBr) 3060, 2940, 2860, 1590, 1495, 1380, 1225, 1135, 1020, 1005, 935, 880, 830, 780, and 740  $cm^{-1}$ ;  $^1H$ -NMR ( $\delta$ ) 6.46-6.75 (m, 8H, ArH,  $H_1$ - $H_4$  and  $H_6$  and  $H_9$ ), 1.58 (broad, 4H,  $H_1$  and  $H_m$ ), 2.36-2.51 (m, 12H,  $H_a$ ,  $H_b$ ,  $H_c$ ,  $H_d$ ,  $H_e$  and  $H_n$ ), 3.42 (broad, 3H,  $H_k$ , and  $H_g$ ), and 3.58-3.63 (t, 2H,  $H_f$ ,  $J = 7$  Hz);  $^{13}C$ -NMR 111.39 ( $C_1$  and  $C_9$ ), 115.26 ( $C_4$  and  $C_6$ ), 120.64 ( $C_3$  and  $C_7$ ), 123.61 ( $C_2$  and  $C_8$ ), 133.30 ( $C_{10}$  and  $C_{10'}$ ), 144.95 ( $C_5$  and  $C_{5'}$ ), 22.28 ( $C_{11}$  and  $C_{11'}$ ), 23.72 ( $C_{12}$ ), 43.60 ( $C_a$  and  $C_b$ ), 53.11 ( $C_c$  and  $C_d$ ), 57.38 ( $C_k$ ), 57.96 ( $C_e$ ) and 59.76 ( $C_f$ ); EIMS (m/z) 367 ( $M^+$ ).

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EXAMPLE 14

1                   10-(4'-N-pyrrolidinobutyl)phenoxazine. The  
experimental steps used for Example 2 were repeated  
using 1g of the product of Example 8, 1.5g KI, 2g K<sub>2</sub>CO<sub>3</sub>  
5 and 1.04g (14.6 mmol, 1.22 mL) of pyrrolidine as  
reactants. The product was chromatographed on silica  
gel with CHCl<sub>3</sub>-MeOH (1:1) to give the free amine as a  
brown oil. An ether solution of this oil was treated  
with ethereal hydrogen chloride to secure the pure  
10 (0.9g) hydrochloride salt. UV- $\lambda_{max}$  (MeOH) 205, 211, 238  
and 320 nm; IR (KBr) 3060, 2840, 1590, 1495, 1380, 1295,  
1270, 1160, 1090, 1045, 915, 840, 830, 795, and 740 cm<sup>-1</sup>;  
<sup>1</sup>H-NMR (' $\delta$ ') 6.43-6.79 (m, 8H, ArH, H<sub>1</sub>-H<sub>4</sub> and H<sub>6</sub>-H<sub>9</sub>),  
1.64-2.10 (m, 8H, H<sub>1</sub>, H<sub>m</sub>, H<sub>o</sub> and H<sub>d</sub>), 2.97-3.17 (m, 6H,  
H<sub>a</sub>, H<sub>b</sub> and H<sub>n</sub>), 3.45-3.54 (m, 2H, H<sub>k</sub>) and 10.10 (H<sup>+</sup>);  
15 <sup>13</sup>C-NMR 111.43 (C<sub>1</sub> and C<sub>9</sub>), 115.41 (C<sub>4</sub> and C<sub>6</sub>), 121.01  
(C<sub>3</sub> and C<sub>7</sub>), 123.73 (C<sub>2</sub> and C<sub>8</sub>), 132.89 (C<sub>1</sub> and C<sub>9</sub>),  
144.87 (C<sub>4</sub> and C<sub>6</sub>), 22.47 (C<sub>o</sub> and C<sub>d</sub>), 23.27 (C<sub>1</sub> and  
C<sub>m</sub>), 43.14 (C<sub>a</sub> and C<sub>b</sub>), 53.50 (C<sub>n</sub>), and 54.91 (C<sub>k</sub>); EIMS  
20 (m/z) 308 (M<sup>+</sup>).

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EXAMPLE 15

1                   10-(chloroac tyl)phenoxazine. To a solution  
of 5g (0.03 mol) of phenoxazine dissolved in 100 mL  
anhydrous acetonitrile containing 10 mL of anhydrous  
5 ether, was added dropwise 7 mL (9.926 g, 0.088 mol) of  
chloroacetyl-chloride with constant stirring. The  
reaction mixture was stirred at room temperature for 5H  
when white crystalline solid separated out (TLC, system  
A,  $R_f=0.030$ ). The crystals were filtered, washed  
10 several times with petroleum ether-ether mixture (9:1)  
and dried under high vacuum to get 6.03g of the product.  
UV- $\lambda_{max}$  (MeOH) 218, 249, and 287 nm; IR (KBr) 3070,  
1675, 1580, 1480, 1410, 1350, 1260, 1210, 1115, 1040,  
860, 815, 750 and 660  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\delta$ ) 7.55-7.61 (m,  
2H, ArH,  $\text{H}_1$  and  $\text{H}_9$ ), 7.12-7.25 (m, 6H, ArH,  $\text{H}_2$ - $\text{H}_4$  and  
15  $\text{H}_6$ - $\text{H}_8$ ), 4.32 (s, 2H,  $\text{H}_1$ );  $^{13}\text{C-NMR}$  110.04 ( $\text{C}_1$  and  $\text{C}_9$ ),  
117.11 ( $\text{C}_4$  and  $\text{C}_6$ ), 123.75 ( $\text{C}_3$  and  $\text{C}_7$ ), 124.32 ( $\text{C}_2$  and  
 $\text{C}_8$ ), 127.60 ( $\text{C}_1$  and  $\text{C}_9$ ), 150.95 ( $\text{C}_4$  and  $\text{C}_6$ ), 41.51  
( $\text{C}_1$ ), and 170 ( $\text{C}_x$ ); EIMS (m/z) 259 ( $\text{M}^+$ ).

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EXAMPLE 16

1           10-(diethylaminoacetyl)phenoxazine. 1g (3.9  
mmol) of the product of Example 15 was dissolved in 150  
mL of anhydrous acetonitrile and 1.5g of KI and 1.13 g  
5 (15.45 mmol, 1.6 mL) of N,N-diethylamine were added to  
it. The reaction mixture was refluxed for 1h when  
substantial amount of the product was formed (TLC,  
system B,  $R_f=0.40$ ). The mixture was processed as in  
Example 2 to get a white crystalline solid which was  
10 further recrystallized in ethylacetate and petroleum  
ether mixture to get the pure compound (0.86g). UV- $\lambda_{max}$   
(MeOH) 220, 246, and 287 nm; IR (KBr) 2800, 1685, 1580,  
1480, 1320, 1210, 1150, 1060, 1035, 940, 860, 810, 755  
and 670  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (' $\delta$ ') 7.53-7.59 (m, 2H, ArH,  $\text{H}_1$  and  
15  $\text{H}_9$ ), 7.05-7.20 (m, 6H, ArH,  $\text{H}_2$ - $\text{H}_4$  and  $\text{H}_6$ - $\text{H}_8$ ), 0.95 (t,  
6H,  $\text{H}_c$  and  $\text{H}_d$ ,  $J=7$  Hz), 2.60 (q, 4H,  $\text{H}_a$  and  $\text{H}_b$ ), and  
3.55 (s, 2H,  $\text{H}_1$ );  $^{13}\text{C-NMR}$  116.79 ( $\text{C}_1$  and  $\text{C}_9$ ), 123.31 ( $\text{C}_4$   
and  $\text{C}_6$ ), 125.02 ( $\text{C}_3$  and  $\text{C}_7$ ), 126.82 ( $\text{C}_2$  and  $\text{C}_8$ ), 129.62  
( $\text{C}_1$  and  $\text{C}_9$ ), 151.07 ( $\text{C}_4$  and  $\text{C}_6$ ), 12.08 ( $\text{C}_c$  and  $\text{C}_d$ ),  
20 47.04 ( $\text{C}_a$  and  $\text{C}_b$ ), 54.99 ( $\text{C}_1$ ), and 169.84 ( $\text{C}_k$ ); MS (m/z)  
296 ( $\text{M}^+$ ).

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EXAMPLE 17

1                   10-(N-morpholinoacetyl)phenoxazine. The same  
procedure used for Example 16 was employed with 1g of  
the product of Example 15, 1.5g KI and 1.347g (16 mmol,  
5 1.4 mL) of morpholine. The solid product was  
recrystallized in a mixture of ethylacetate, petroleum  
ether and ether and the free base was converted into  
hydrochloride salt (1.07g) using ethereal hydrochloride.  
UV- $\lambda_{max}$  213, 246, and 287 nm; IR (KBr) 2980, 2860, 1690,  
10 1485, 1440, 1355, 1270, 1180, 1120, 1070, 1005, 900,  
870, 855, 760 and 640  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (' $\delta$ ') 7.60 (broad,  
2H, ArH,  $\text{H}_1$  and  $\text{H}_9$ ), 7.12-7.34 (m, 6H, ArH,  $\text{H}_2$ - $\text{H}_4$  and  
 $\text{H}_6$ - $\text{H}_8$ ), 2.40-2.60 (t, 6H,  $\text{H}_a$  and  $\text{H}_b$ ,  $J=12$  Hz), 3.35 (s,  
2H,  $\text{H}_1$ ) and 3.50-3.70 (t, 4H,  $\text{H}_c$  and  $\text{H}_d$ );  $^{13}\text{C-NMR}$  117.03  
15 ( $\text{C}_1$  and  $\text{C}_9$ ), 123.90 ( $\text{C}_4$  and  $\text{C}_6$ ), 124.98 ( $\text{C}_3$  and  $\text{C}_7$ ),  
126.95 ( $\text{C}_2$  and  $\text{C}_8$ ), 127.91 ( $\text{C}_1$  and  $\text{C}_9$ ), 150.54 ( $\text{C}_4$   
and  $\text{C}_6$ ), 52.41 ( $\text{C}_a$  and  $\text{C}_b$ ), 57.01 ( $\text{C}_1$ ), 63.23 ( $\text{C}_c$  and  
 $\text{C}_d$ ), and 163.40 ( $\text{C}_x$ ); MS ( $m/z$ ) 310 ( $\text{M}^+$ ).

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EXAMPLE 18

1                   10-(N-piperidinoacetyl)phenoxazine. The  
method employed for Example 17 was used with 1g of the  
product of Example 15, 1.5g KI and 1.31g (15.4 mmol,  
5 1.52 mL) of piperidine to get 0.95g of the title  
compound. UV- $\lambda_{max}$  (MeOH) 218, 246 and 287 nm; IR (KBr)  
2960, 1670, 1610, 1580, 1480, 1370, 1330, 1260, 1190,  
1120, 1040, 940, 890, 855, 810, 765, and 655  $\text{cm}^{-1}$ ;  $^1\text{H}$ -  
NMR ( $\delta$ ) 7.57-7.61 (m, 2H, ArH, H<sub>1</sub> and H<sub>9</sub>), 7.12-7.16  
10 (m, 6H, ArH, H<sub>2</sub>-H<sub>4</sub> and H<sub>6</sub>-H<sub>8</sub>), 1.51 (very broad, 6H, H<sub>2</sub>,  
H<sub>4</sub> and H<sub>6</sub>), 2.44 (m, 4H, H<sub>a</sub> and H<sub>b</sub>) and 3.34 (s, 2H,  
H<sub>1</sub>);  $^{13}\text{C}$ -NMR 116.72 (C<sub>1</sub> and C<sub>9</sub>), 123.28 (C<sub>4</sub> and C<sub>6</sub>),  
124.97 (C<sub>3</sub> and C<sub>7</sub>), 126.79 (C<sub>2</sub> and C<sub>8</sub>), 129.48 (C<sub>1</sub> and  
C<sub>9</sub>), 151.01 (C<sub>4</sub> and C<sub>6</sub>), 23.92 (C<sub>2</sub>), 25.93 (C<sub>3</sub> and  
15 C<sub>7</sub>), 54.15 (C<sub>a</sub> and C<sub>b</sub>), 60.80 (C<sub>1</sub>), and 168.92 (C<sub>x</sub>);  
EIMS (m/z) 308 (M<sup>+</sup>).

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EXAMPLE 19

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## 10-(8-hydroxyethylpiperazinoacetyl)

phenoxazine. The procedure used for Example 17 was repeated with 1g of the product of Example 15, 1.5g KI and 2g (15.4 mmol, 1.9 mL) of 8-hydroxyethylpiperazine. 5 Recrystallization of the white solid yielded 1.17 g of the title compound.  $UV_{max}$  (MeOH) 213, 246 and 287 nm; IR (KBr) 3200, 2940, 1685, 1665, 1480, 1265, 1190, 1160, 945, 855, 765 and 640  $cm^{-1}$ ;  $^1H$ -NMR (' $\delta$ ') 7.53-7.58 (m, 2H, ArH,  $H_1$  and  $H_9$ ), 7.08-7.25 (m, 6H, ArH,  $H_2$ - $H_4$  and  $H_6$ - $H_8$ ), 2.48 (m, 1OH,  $H_a$ ,  $H_b$ ,  $H_c$ ,  $H_d$  and  $H_e$ ), 2.70 (s, 1H,  $H_g$ , disappearing on  $D_2O$  exchange), 3.39 (s, 2H,  $H_1$ ) and 3.60 (t, 2H,  $H_f$ ,  $J=7$  Hz);  $^{13}C$ -NMR 116.85 ( $C_1$  and  $C_9$ ), 123.34 ( $C_4$  and  $C_6$ ), 124.86 ( $C_3$  and  $C_7$ ), 126.99 ( $C_2$  and  $C_8$ ), 129.25 ( $C_{10}$  and  $C_{10'}$ ), 151.04 ( $C_5$  and  $C_{5'}$ ), 15 52.70 ( $C_a$  and  $C_b$ ), 52.90 ( $C_c$  and  $C_d$ ), 57.70 ( $C_g$ ), 59.23 ( $C_1$ ), 59.80 ( $C_f$ ), and 168.43 ( $C_k$ ); EIMS (m/z) 353 ( $M^+$ ).

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EXAMPLE 20

1                   10-(N-pyrrolidinoacetyl)phenoxazine. The  
experimental procedure used for Example 17 was employed  
with 1g of the product of Example 15, 1.5g KI and 1.095g  
5 (15.4 mmol, 1.3 mL) of pyrrolidine. Purification by  
recrystallization afforded 1.02 g of the title compound.  
UV- $\lambda_{max}$  (MeOH) 214, 240, and 286 nm; IR (KBr) 2980,  
2820, 1695, 1670, 1480, 1455, 1340, 1270, 1180, 1100,  
1040, 985, 905, 855, 755 and 640  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (' $\delta$ ')  
10 7.58-7.63 (m, 2H, ArH,  $\text{H}_1$  and  $\text{H}_9$ ), 7.07-7.18 (m, 6H,  
ArH,  $\text{H}_2$ - $\text{H}_4$  and  $\text{H}_6$ - $\text{H}_8$ ), 1.77 (t, 4H,  $\text{H}_c$  and  $\text{H}_d$ ,  $J=7$  Hz),  
2.64 (t, 4H,  $\text{H}_a$  and  $\text{H}_b$ ) and 3.51 (s, 2H,  $\text{H}_1$ );  $^{13}\text{C-NMR}$   
116.80 ( $\text{C}_1$  and  $\text{C}_9$ ), 123.33 ( $\text{C}_4$  and  $\text{C}_6$ ), 125.06 ( $\text{C}_3$  and  
 $\text{C}_7$ ), 126.85 ( $\text{C}_2$  and  $\text{C}_8$ ), 129.28 ( $\text{C}_1$  and  $\text{C}_9$ ), 151.00  
15 ( $\text{C}_4$  and  $\text{C}_6$ ), 23.73 ( $\text{C}_c$  and  $\text{C}_d$ ), 53.83 ( $\text{C}_a$  and  $\text{C}_b$ ),  
57.24 ( $\text{C}_1$ ), and 168.92 ( $\text{C}_k$ ); EIMS ( $m/z$ ) 294 ( $\text{M}^+$ ).

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EXAMPLE 21

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10-(trifluoroacetyl)phenoxazine. To a solution of 200 mg of phenoxazine in 10 mL anhydrous chloroform and 4 mL anhydrous ether, was added 50  $\mu$ l of (0.7435g, 3.54 mmol) trifluoroacetic anhydride. The resulting mixture was stirred at room temperature for 8 hours. The formation of the product was monitored by TLC (system A). The product solution was then extracted with chloroform and evaporated. The residue was subjected to column chromatography which afforded the pure title compound. UV- $\lambda_{max}$  (MeOH) 212, 238, and 252 nm; IR (KBr) 3375, 1695, 1580, 1480, 1455, 1390, 1290, 1170, 1110, 1030, 965, 890, 850, 800, 760, 730, and 670  $cm^{-1}$ ;  $^1H$ -NMR ( $\delta$ ) 7.57-7.61 (m, 2H, ArH, H<sub>1</sub> and H<sub>9</sub>), 7.14-7.32 (m, 6H, ArH, H<sub>2</sub>-H<sub>4</sub> and H<sub>6</sub>-H<sub>8</sub>);  $^{13}C$ -NMR 117.20 (C<sub>1</sub> and C<sub>9</sub>), 123.83 (C<sub>4</sub> and C<sub>6</sub>), 124.34 (C<sub>3</sub> and C<sub>7</sub>), 128.34 (C<sub>2</sub> and C<sub>8</sub>), 151.04 (C<sub>1</sub> and C<sub>9</sub>, and C<sub>4</sub> and C<sub>6</sub>), and >200 ppm (C<sub>x</sub> and C<sub>1</sub>); EIMS (m/z) 279 (M<sup>+</sup>).

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TABLE I

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PHYSICAL PROPERTIES OF N-(ALKYLAMINO) OR N-ACYLAMINO DERIVATIVES OF PHENOXAZINE		
Product Of Example No.	Yield, %	mp, °C
1	80	53
2	70	ND
3	90	83-84
4	80	198*
5	70	202*
6	85	108
7	75	158-159*
8	80	46
9	60	127*
10	80	115*
11	80	89 187*
12	90	190*
13	90	114
14	70	170*
15	85	143-144
16	75	39
17	80	130*
18	80	110-111
19	85	70-71
20	80	96-98*
21	70	90
* - HCl salt		

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1           The potentiating agent is preferably  
administered by infusion in solution in sterile water.  
The potentiating agents as hydrochloride salts can be  
dissolved in sterile water. The agents as bases can be  
5           solubilized in 1N hydrochloric acid, following which the  
solution is back titrated with sodium hydroxide to  
provide a final pH between 7 and 8.

Cytotoxic agents whose cytotoxicity would be  
potentiated by agents within the scope of this invention  
include VCR, VLB, doxorubicin, colchicine, actinomycin  
10          D, daunomycin, M-AMSA, and other anthracyclic compounds.

The potentiating agent is administered to  
tumor cells which are exposed to one or more cytotoxic  
agents. By "exposed" is meant that the cytotoxic agent  
has been administered simultaneously with the  
15          potentiating agent, and/or is administered subsequently  
to the administration of the potentiating agent, so long  
as at least some of the cytotoxic agent(s) is present in  
the tumor cell when the potentiating agent is present in  
the tumor cell. The cytotoxic agent should not be  
20          administered before the potentiating agent. Preferably,  
the cytotoxic agent is administered when the  
potentiating agent concentration reaches steady state  
during administration by infusion.

It will be recognized that the amount of  
25          potentiating agent to be administered will vary between  
hosts, between cytotoxic agents and between potentiating  
agents, but the effective amounts can readily be  
ascertained by those of ordinary skill in this field.  
As guidance one can refer to the data in Examples 22-24  
30          as well as the following Table. In general, though,  
effective amounts to potentiate cytotoxic agents are

1 about 2000-3000 moles of potentiating agent per mole of  
VCR; about 1,000-2,000 moles of potentiating agent per  
mole of VLB; and about 25-35 moles of potentiating agent  
per mole of VP-16 (Etoposide). These values, and the  
5 corresponding values for any other cytotoxic agents, can  
readily be converted if desired into dosages per host  
body weight by calculation based on the dosages for the  
cytotoxic agent of interest. The in vitro techniques  
described herein can be employed to determine the  
10 effectiveness of any particular potentiating agent with  
any given cytotoxic agent or agents.

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EXAMPLE 22

1                   Table II b low gives r presentativ in vivo  
values of the molar ratios (shown below as "compound:  
(cytotoxic agent)") of potentiating agent to cytotoxic  
5 agent for compounds within the scope of this invention.  
Vincristine (VCR) was administered to mice at 3 mg/kg  
(3.25  $\mu$ mol/kg); vinblastine (VLB) was at 5 mg/kg (5.5  
 $\mu$ mol/kg); VP-16 (Etoposide) was at 50 mg/kg/day for 3  
days (0.255 mmol/kg total). The compound number is the  
10 number of the example in which the potentiating agent  
was prepared.

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TABLE II

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Compound No.	Compound:VCR	Compound:VLB	Compound:VP-16
3	2345	1388	29.9
4	2483	1469	31.6
11	2375	1405	30.3
18	2498	1478	31.8

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EXAMPLE 23Evaluation of N-substituted  
Phenoxazines For Anti-MDR activity

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5 A cloned line of human colon adenocarcinoma, GC<sub>3</sub>/Cl<sup>31</sup>, which is intrinsically resistant to VCR ( $\approx$  4-fold relative to KB-3-1), was routinely grown at 37°C in antibiotic-free RPMI-1640 medium supplemented with 2 mM glutamine and 10% FBS (Hyclone Laboratories, Inc., Logan, UT) in a humidified atmosphere of 5% CO<sub>2</sub> and 95%  
10 air. Human epidermoid carcinoma KB-3-1 cells and a colchicine selected MDR variant, KBCh<sup>R</sup>-8-5, were obtained which was cross-resistant to VCR (45-fold) and VLB (6.3-fold); it was grown in monolayer culture at 37°C in DMEM with 10% FBS and L-glutamine in a  
15 humidified atmosphere of 10% CO<sub>2</sub> in air. The resistance of the KBCh<sup>R</sup>-8-5 cells was maintained by culturing them with colchicine (10 ng/ml).

Then, 2 mL of cell suspensions ( $2 \times 10^6$ ) were plated in 35 x 10 mm style "easy grip" culture dishes (Becton Dickinson Co., Lincoln Park, NJ). Cells were  
20 allowed to attach to plastic overnight at 37°C. Medium was aspirated and cells were washed with (2 x 2 mL) physiologic tris (PT) buffer. Monolayers were incubated at room temperature for 10 minutes in PT buffer prior to aspiration and adding 1 mL of serum-free RPMI-1640 Hepes  
25 buffer (10.4g RPMI-1640 medium in 1L of 25 mM Hepes, pH 7.4) containing 70.4 nM [<sup>3</sup>H] VCR (sp.act. 7.1 Ci/mmol) or 49.5 nM [<sup>3</sup>H] VLB (sp. act. 10.1 Ci/mmol) with or without a compound of Examples 1-21 (100  $\mu$ M) or VRP dissolved in H<sub>2</sub>O dissolved in DMSO (final culture  
30 concentration <0.1% DMSO). After 2h of incubation at room temperature, medium was rapidly aspirated to

1 terminate drug accumulation, and monolayers were washed  
four times with ice-cold PBS (g/L: NaCL 8.0;  
Na<sub>2</sub>HPO<sub>4</sub>.12H<sub>2</sub>O, 2.9; KCl 0.2; KH<sub>2</sub>PO<sub>4</sub>, 0.2) and drained.  
To each dish, 1 ml of trypsin-EDTA (0.05% trypsin, 0.53  
5 mM EDTA) was added. After 1 minute, monolayers were  
triturated to give a uniform suspension of cells, and  
radioactivity in 0.75 ml was determined by scintillation  
counting. Cell number per dish was determined on 200 µl  
of suspension using the method of Butler, and amounts of  
intracellular VCR or VLB were determined. The results  
10 are set forth in Table III, in which the compound number  
is the number of the Example in which the compound (or  
"modulator" or "potentiating agent") was prepared.

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TABLE III

1	<b>EFFECTS OF N-SUBSTITUTED PHENOXAZINES ON MDR ACTIVITY</b>				
	<b>Vinca Accumulation <sup>a</sup>(% control)</b>				
	<b>KB Ch<sup>a</sup>-8-5 Cells</b>		<b>GC<sub>3</sub>/Cl Cells</b>		
5	<b>Modulator Compound Number</b>	<b>VCR</b>	<b>VLB</b>	<b>VCR</b>	<b>VLB</b>
	1	454	342	846	570
	2	546	2123	439	1025
10	3	473	1666	464	1070
	4	742	1717	634	960
	5	435	1227	282	633
	6	343	824	368	879
	7	408	969	250	757
15	8	398	792	317	361
	9	211	697	325	737
	10	92	403	382	1165
	11	702	2684	477	1175
	12	196	1071	416	1121
20	13	91	188	543	1340
	14	198	477	412	1315
	15	138	236	171	284
	16	184	953	160	305
	17	290	674	213	298
25	18	326	2023	177	446
	19	280	776	157	426
	20	188	776	151	296
	21	415	827	230	222
	Verapamil	402	1124	178	238
30	<sup>a</sup> $\frac{\text{vinca uptake with modulator}}{\text{vinca uptake without modulator}} \times 100$				
	<sup>b</sup> Compounds were tested at 100μM. All values represent the mean of two separate experiments with a SD of less than 10% of the mean; each experiment was done in triplicate.				



EXAMPLE 24Evaluation of N-substituted Phenoxazines  
Cytotoxicity To Tumor Cells

1  
5 The KBCh<sup>R</sup>-8-5 cells were plated in triplicate  
at a density of 1000 cells per well and GC<sub>3</sub> at 3000  
cells per well in Falcon 6-well flat-bottom tissue  
culture plates (Becton Dickinson Co., Lincoln Park, NJ).  
After 24h, incubation medium was replaced with 3 mL of  
fresh medium containing compounds 1-4 or 10-14 or 18 at  
10 concentrations ranging from 1-100  $\mu$ m (final culture  
concentration, 0.1% DMSO), and cells were incubated at  
37°C for a further 7 days. The medium was aspirated and  
cells were washed once with 2 mL of 0.9% saline and  
dried overnight. Colonies were stained with 1 mL of  
15 0.1% crystal violet followed by washing twice with  
distilled water and were counted using an automated  
ARTEK Model 880 colony counter. The IC<sub>50</sub> values were  
determined from concentration-percent-cell-survival  
curves and were defined as the concentrations of  
phenoxazines required for 50% reduction in colonies  
20 compared to controls. The results of these measurements  
are set forth in Table IV.

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TABLE IV

CYTOTOXICITY OF N-SUBSTITUTED PHENOXAZINES		
IC <sub>50</sub> , <sup>a</sup> $\mu$ M		
Compound Number	KBCh <sup>R</sup> -8-5	GC <sub>3</sub> /CL
1	57	83.00
2	15	ND
3	38	37
4	73	40
10	<10	16
11	18	27
12	<10	7
13	<10	7
14	<10	8
18	73	ND
<sup>a</sup> IC <sub>50</sub> is the concentration required to produce 50% reduction in clonogenic survival of GC <sub>3</sub> /CL and KBCh <sup>R</sup> -8-5 cells under the conditions described in Example 23.		

EXAMPLE 25

1

Effect Of N-substituted Phenoxazines  
On In Vitro Cytotoxicity Of VLB And VCR

Tumor cells were treated with graded  
5 concentrations of VCR and VLB in the absence or presence  
of nontoxic concentrations of the products of Examples  
1, 3, 4 and 18. The plates were then transferred to a  
CO<sub>2</sub> incubator and, after further incubation for 7 days  
at 37°C, colonies were enumerated as described in  
10 Example 23. The results are set forth in Table V.

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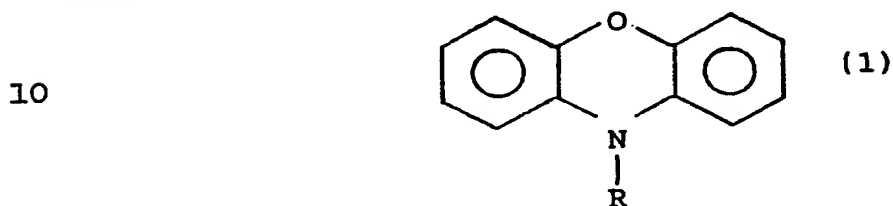
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TABLE V

Potentiation Of Cytotoxicity Of Vincristine And Vinblastine By N-substituted Phenoxazines Against GC <sub>3</sub> /Cl And KBCh <sup>a</sup> -8-5 Cells					
IC <sub>50</sub> Values, nM					
KB Ch <sup>a</sup> -8-5 Cells			GC <sub>3</sub> /Cl Cells		
Compound Number	Concentration of Modulator <sup>a</sup> ( $\mu$ M)	VCR	VLB	VCR	VLB
no modulator	-	32.0	20.0	27.0	7.4
1	50	-	-	9.0	-
3	25	-	2.7	-	2.0
4	25	1.2	1.6	0.85	2.0
18	49	-	2.3	-	2.2
<sup>a</sup> IC <sub>50</sub> concentration of modulator					

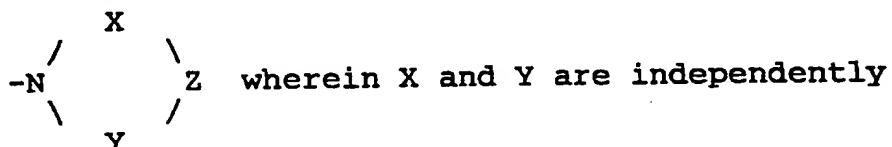
1 WHAT IS CLAIMED IS:

1. A method of potentiating the cytotoxicity of an agent cytotoxic to a tumor cell, comprising administering to said tumor cell, while it is exposed to said cytotoxic agent, a potentiating agent in an amount effective to potentiate the cytotoxicity of said cytotoxic agent to said cell, wherein said potentiating agent comprises a compound of the formula (1):



- or a pharmacologically acceptable salt thereof,  
 15 wherein R is -H or  $-[C(O)]_a-(CH_2)_b-A$ ; wherein a is 0 or 1 and b is an integer from 0 to 6, provided that a and b are not both zero; and

- A is selected from the group consisting of  
 $-NR_1R_2$  wherein  $R_1$  and  $R_2$  are independently  
 20 alkyl having 1 to 4 carbon atoms; and either or both of  $R_1$  and  $R_2$  are optionally substituted with -OH;



- 25 alkylene having 1 to 4 carbon atoms, and Z is -O-,  
 $-N(R_3)-$  or  $-CH(R_4)-$ , wherein  $R_3$  is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group, and wherein  $R_4$  is hydrogen or alkyl  
 30 having 1 to 4 carbon atoms optionally substituted with a hydroxyl group;  
 halide; and trihalomethyl.

1           2. The method of Claim 1 wherein said tumor  
cell is present in a living host.

          3. The method of Claim 1 wherein said  
cytotoxic agent is selected from the group consisting of  
5 vincristine, vinblastine, etoposide, doxorubicin,  
colchicine, actinomycin D, daunomycin, m-AMSA, and  
mixtures thereof.

          4. The method of Claim 1 wherein said tumor  
cell exhibits multiple drug resistance.

10           5. The method of Claim 1 wherein a is zero; b  
is 3 or 4; R<sub>1</sub> and R<sub>2</sub> are independently selected from the  
group consisting of ethyl, propyl, ω-hydroxyethyl, and  
ω-hydroxypropyl; X and Y are each independently selected  
from the group consisting of -CH<sub>2</sub>- and -CH<sub>2</sub>CH<sub>2</sub>-; and R<sub>3</sub>  
15 and R<sub>4</sub> are independently selected from the group  
consisting of -H, ethyl, propyl, ω-hydroxyethyl, and ω-  
hydroxypropyl.

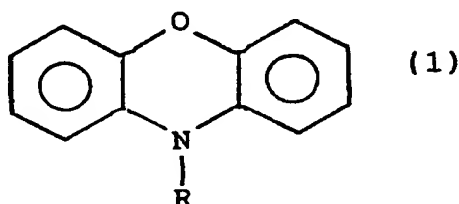
          6. The method of Claim 5 wherein said  
potentiating agent is 10-(3'-chloropropyl)-phenoxazine,  
10-(3'-diethylaminopropyl)-phenoxazine, 10-(3'-  
20 bishydroxyethylaminopropyl)-phenoxazine, 10-(3'-N-  
morpholinopropyl)-phenoxazine, 10-(3'-N-  
piperidinopropyl)-phenoxazine, 10-(3'-β-  
hydroxyethylpiperazinopropyl)-phenoxazine, (10-(3'-N-  
pyrrolidinopropyl)-phenoxazine, 10-(4'-chlorobutyl)-  
25 phenoxazine, 10-(4'-diethylaminobutyl)-phenoxazine, 10-  
(4'-bishydroxyethylaminobutyl)-phenoxazine, 10-(4'-N-  
morpholinobutyl)-phenoxazine, 10-(4'-piperidinobutyl)-  
phenoxazine, 10-(4'-β-hydroxyethylpiperazinobutyl)-  
phenoxazine, 10-(4'-N-pyrrolidinobutyl)-phenoxazine or  
30 pharmacologically acceptable salts thereof.

          7. The method of Claim 1 wherein a is 1.

8. The method of Claim 7 wherein b is 1 or 2;  
1 R<sub>1</sub> and R<sub>2</sub> are independently selected from the group  
consisting of ethyl, propyl, ω-hydroxyethyl, and ω-  
hydroxypropyl; X and Y are each independently selected  
5 from the group consisting of -CH<sub>2</sub>- and -CH<sub>2</sub>CH<sub>2</sub>-; and R<sub>3</sub>  
and R<sub>4</sub> are independently selected from the group  
consisting of -H, ethyl, propyl, ω-hydroxyethyl, and ω-  
hydroxypropyl.

9. The method of Claim 8 wherein said  
10 potentiating agent is 10-(chloroacetyl)-phenoxazine, 10-  
(diethylaminoacetyl)-phenoxazine, 10-(N-  
morpholinoacetyl)-phenoxazine, 10-(N-piperidinoacetyl)-  
phenoxazine, 10-(β-hydroxyethylpiperazinoacetyl)-  
phenoxazine, 10-(N-pyrrolidinoacetyl)-phenoxazine, 10-  
15 (trifluoroacetyl)-phenoxazine or pharmacologically  
acceptable salts thereof.

10. A composition comprising a cytotoxic  
agent toxic to tumor cells, and a potentiating agent  
which potentiates the cytotoxicity of said cytotoxic  
agent, wherein said potentiating agent comprises a  
20 compound of the formula (1)



25

or a pharmacologically acceptable salt thereof,  
wherein R is -H or -[C(O)]<sub>a</sub>-(CH<sub>2</sub>)<sub>b</sub>-A;  
wherein a is 0 or 1 and b is an integer from 0 to 6,  
30 provided that a and b are not both zero; and  
A is selected from the group consisting of

1            $-NR_1R_2$  wherein  $R_1$  and  $R_2$  are independently  
alkyl having 1 to 4 carbon atoms, and either or both of  
 $R_1$  and  $R_2$  are optionally substituted with  $-OH$ ;

5           
$$\begin{array}{c} \text{X} \\ \diagup \quad \diagdown \\ -N \quad \quad Z \\ \diagdown \quad \diagup \\ \text{Y} \end{array}$$
 wherein X and Y are independently

alkylene having 1 to 4 carbon atoms, and Z is  $-O-$ ,  $-N(R_3)-$  or  $-CH(R_4)-$ , wherein  $R_3$  is hydrogen or alkyl  
10 having 1 to 4 carbon atoms optionally substituted with a  
hydroxyl group, and wherein  $R_4$  is hydrogen or alkyl  
having 1 to 4 carbon atoms optionally substituted with a  
hydroxyl group;

halide; and trihalomethyl;

15           wherein said cytotoxic agent and potentiating  
agent are present in amounts effective to render the  
composition cytotoxic to tumor cells.

11. The composition of Claim 10 wherein said  
cytotoxic agent is selected from the group consisting of  
vincristine, vinblastine, etoposide, doxorubicin,  
20 colchicine, actinomycin D, daunomycin, m-AMSA, and  
mixtures thereof.

12. The composition of Claim 10 wherein a is  
zero; b is 3 or 4;  $R_1$  and  $R_2$  are independently selected  
from the group consisting of ethyl, propyl,  $\omega$ -  
25 hydroxyethyl, and  $\omega$ -hydroxypropyl; X and Y are each  
independently selected from the group consisting of  $-CH_2-$   
and  $-CH_2CH_2-$ ; and  $R_3$  and  $R_4$  are independently  
selected from the group consisting of  $-H$ , ethyl, propyl,  
 $\omega$ -hydroxyethyl, and  $\omega$ -hydroxypropyl.

30           13. The composition of Claim 12 wherein said  
potentiating agent is 10-(3'-chloropropyl)-phenoxazine,



1 10-(3'-diethylaminopropyl)-phenoxazine, 10-(3'-  
bishydroxyethylaminopropyl)-phenoxazine, 10-(3'-N-  
morpholinopropyl)-phenoxazine, 10-(3'-N-  
piperidinopropyl)-phenoxazine, 10-(3'-β-  
5 hydroxyethylpiperazinopropyl)-phenoxazine, (10-(3'-N-  
pyrrolidinopropyl)-phenoxazine, 10-(4'-chlorobutyl)-  
phenoxazine, 10-(4'-diethylaminobutyl)-phenoxazine, 10-  
(4'-bishydroxyethylaminobutyl)-phenoxazine, 10-(4'-N-  
morpholinobutyl)-phenoxazine, 10-(4'-piperidinobutyl)-  
10 phenoxazine, 10-(4'-β-hydroxyethylpiperazinobutyl)-  
phenoxazine, 10-(4'-N-pyrrolidinobutyl)-phenoxazine or  
pharmacologically acceptable salts thereof.

14. The composition of Claim 10 wherein a is  
1; b is 1 or 2; R<sub>1</sub> and R<sub>2</sub> are independently selected  
from the group consisting of ethyl, propyl, ω-  
15 hydroxyethyl, and ω-hydroxypropyl; wherein X and Y are  
each independently selected from the group consisting of  
-CH<sub>2</sub>- and -CH<sub>2</sub>CH<sub>2</sub>-; and R<sub>3</sub> and R<sub>4</sub> are independently  
selected from the group consisting of -H, ethyl, propyl,  
ω-hydroxyethyl, and ω-hydroxypropyl.

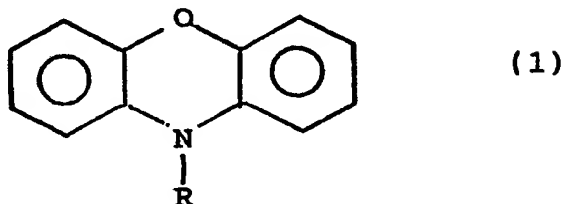
20 15. The composition of Claim 14 wherein said  
potentiating agent is 10-(chloroacetyl)-phenoxazine, 10-  
(diethylaminoacetyl)-phenoxazine, 10-(N-  
morpholinoacetyl)-phenoxazine, 10-(N-piperidinoacetyl)-  
phenoxazine, 10-(β-hydroxyethylpiperazinoacetyl)-  
25 phenoxazine, 10-(N-pyrrolidinoacetyl)-phenoxazine, 10-  
(trifluoroacetyl)-phenoxazine or pharmacologically  
acceptable salts thereof.

16. A method of killing a tumor cell which  
comprises administering to said cell a composition  
30 according to Claim 10 in an amount effective to kill  
said cell.

17. The method of Claim 16 wherein said tumor cell is present in a living host.

18. The method of Claim 16 wherein said tumor cell exhibits multiple drug resistance.

19. A compound of the formula (1)



and pharmacologically acceptable salts thereof, wherein R is  $-[C(O)]_a-(CH_2)_b-A$ ; wherein a is 0 or 1 and b is an integer from 0 to 6, provided that a and b are not both zero; and

A is selected from the group consisting of  $-NR_1R_2$  wherein  $R_1$  and  $R_2$  are independently alkyl having 1 to 4 carbon atoms, and either or both of  $R_1$  and  $R_2$  are optionally substituted with -OH;

20 
$$\begin{array}{c} \text{X} \\ / \quad \backslash \\ -N \quad \quad Z \\ \backslash \quad / \\ \text{Y} \end{array}$$
 wherein X and Y are independently

alkylene having 1 to 4 carbon atoms, and Z is -O-, -N( $R_3$ )- or -CH( $R_4$ )-, wherein  $R_3$  is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group, and wherein  $R_4$  is hydrogen or alkyl having 1 to 4 carbon atoms optionally substituted with a hydroxyl group;

30 halide; and trihalomethyl.

20. A compound or salt according to Claim 19 wherein a is zero; b is 3 or 4;  $R_1$  and  $R_2$  are

1 independently selected from the group consisting of  
ethyl, propyl,  $\omega$ -hydroxyethyl, and  $\omega$ -hydroxypropyl; X  
and Y are each independently selected from the group  
consisting of  $-\text{CH}_2-$  and  $-\text{CH}_2\text{CH}_2-$ ; and  $\text{R}_3$  and  $\text{R}_4$  are  
5 independently selected from the group consisting of  $-\text{H}$ ,  
ethyl, propyl,  $\omega$ -hydroxyethyl, and  $\omega$ -hydroxypropyl.

21. The compound according to Claim 20 which  
is 10-(3'-chloropropyl)-phenoxazine, 10-(3'-  
diethylaminopropyl)-phenoxazine, 10-(3'-  
bishydroxyethylaminopropyl)-phenoxazine, 10-(3'-N-  
10 morpholinopropyl)-phenoxazine, 10-(3'-N-  
piperidinopropyl)-phenoxazine, 10-(3'- $\beta$ -  
hydroxyethylpiperazinopropyl)-phenoxazine, (10-(3'-N-  
pyrrolidinopropyl)-phenoxazine, 10-(4'-chlorobutyl)-  
phenoxazine, 10-(4'-diethylaminobutyl)-phenoxazine, 10-  
15 (4'-bishydroxyethylaminobutyl)-phenoxazine, 10-(4'-N-  
morpholinobutyl)-phenoxazine, 10-(4'-piperidinobutyl)-  
phenoxazine 10-(4'- $\beta$ -hydroxyethylpiperazinobutyl)-  
phenoxazine, 10-(4'-N-pyrrolidinobutyl)-phenoxazine or  
pharmacologically acceptable salts thereof.

22. A compound or salt according to Claim 19  
wherein a is 1; b is 1 or 2;  $\text{R}_1$  and  $\text{R}_2$  are independently  
selected from the group consisting of ethyl, propyl,  $\omega$ -  
hydroxyethyl, and  $\omega$ -hydroxypropyl; X and Y are each  
independently selected from the group consisting of -  
25  $\text{CH}_2-$  and  $-\text{CH}_2\text{CH}_2-$ ; and  $\text{R}_3$  and  $\text{R}_4$  are independently  
selected from the group consisting of  $-\text{H}$ , ethyl, propyl,  
 $\omega$ -hydroxyethyl, and  $\omega$ -hydroxypropyl.

23. The compound according to Claim 22 which  
is 10-(chloroacetyl)-phenoxazine, 10-  
30 (diethylaminoacetyl)-phenoxazine, 10-(N-  
morpholinoacetyl)-phenoxazine, 10-(N-piperidinoacetyl)-

1 phenoxazine, 10-(8-hydroxyethylpiperazinoac tyl)-  
ph noxazin , 10-(N-pyrrolidinoac tyl)-phenoxazin , 10-  
(trifluoroacetyl)-phenoxazine or pharmacologically  
acceptable salts thereof.

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/06681

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl.5                      A 61 K 31/535                      C 07 D 265/38		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.Cl.5	A 61 K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>o</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	Cancer Communications, vol. 2, no. 7, 1990, Pergamon Press, (US), K.N. THIMMAIAH et al.: "Structural determinants of phenoxazine type compounds required to modulate the accumulation of vinblastine and vincristine in multidrug-resistant cell lines", pages 249-259, see abstract; page 249; page 251, table 1; pages 257-258 <div style="text-align: center;">---</div>	1-4, 10, 11, 16- 18
Y	<div style="text-align: center;">---</div> <div style="text-align: center;">-/-</div>	5-9
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>o</sup> Special categories of cited documents : <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
16-11-1992		07. 12. 92
International Searching Authority		Signature of Authorized Officer
EUROPEAN PATENT OFFICE		 Mme Dagmar FRANK

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		Relevant to Claim No.
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	
X	Gann, The Japanese Journal of Cancer Research, vol. 64, no. 4, August 1973, F. KANZAWA et al.: "Antitumor activity of haloacetylcarbazole derivatives", pages 391-396, see pages 392-393, tables I+II; page 394 ---	1-2,7,9 ,19,23
X	GB,A, 850334 (CHAS. PFIZER & CO. INC.) 5 October 1960, see pages 1-3; claims 10-12,14 ---	19-21
Y	---	5-9
X	BE,A, 569697 (S.A. RECHERCHE ET INDUSTRIE THERAPEUTIQUES) 24 January 1959, see pages 4,8; claims 9,10,18,22,23 ---	19-21
Y	---	5-9
T	Journal of Medicinal Chemistry, vol. 35, no. 18, 4 September 1992, American Chemical Society, K.N. THIMMAIAH et al.: "Synthesis and chemical characterization of N-substituted phenoxazines directed toward reversing vinca alkaloid resistance in multidrug-resistant cancer cells", pages 3358-3364, see whole article -----	1-23

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

**US 9206681  
SA 63482**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on 27/11/92  
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A- 850334		None	
BE-A- 569697		None	